Phloem transport: **Are you chaperoned?**

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Long-distance transport via the vasculature in plants is critical for nutrient dissemination, as well as transport of growth regulatory molecules such as hormones. Evidence is now accumulating that protein and RNA molecules also use this transport pathway, possibly to regulate developmental and physiological processes.

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Multicellular organisms rely on continuous communication between individual cells, both locally and systemically, to coordinate growth in an ever-changing environment. Long-distance signaling in plants is thought to occur predominately through the phloem, a component of the vascular system once assumed to function solely as a conduit for nutrient dissemination (Figure 1a,b). Phloem tissue contains two specialized cell types, companion cells and sieve elements, which derive from division of the same phloem mother cell (Figure 1c). Following division, sieve elements undergo a developmental program whereby their nuclei, vacuoles and most other organelles degenerate, giving rise to cells that lack the capacity for transcription or translation but are highly specialized to deliver sugars, hormones, amino acids, proteins and possibly RNAs to the whole plant [1].

Sieve elements themselves are maintained for months to years by replacement proteins that are imported from adjoining companion cells through plasmodesmata, narrow channels that traverse the cell wall. Plasmodesmata are also used by plant viruses to transport their genomes between cells and, via the phloem, to infect distant tissues. Now, evidence is accumulating that endogenous proteins, RNAs and possibly protein–RNA complexes are transported both from cell to cell and throughout the vascular system. For this long-distance transport, molecules entering the transport stream need to interact with the plasmodesmata between companion cells and sieve elements, either directly or indirectly via plasmodesmata 'chaperones'. An endogenous plant protein that might act as such a chaperone for phloem transport has recently been identified [2]. Such long-distance signaling through the phloem could function in regulating developmental and physiological programs, as well as in the initiation and maintenance of defense responses to pathogen infections.

Companion cell–sieve element plasmodesmata

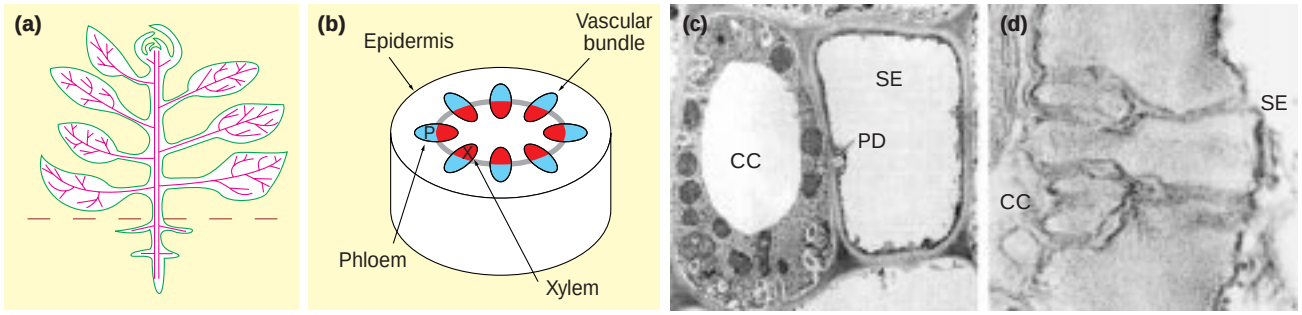
The plasmodesmata that connect companion cells to their dependent sieve elements are unique in structure and function. Instead of being single or minimally branched passages through the cell wall — as found connecting the mesophyll cells that are the principal cell type in the leaf — these plasmodesmata are composed of a network of interconnecting channels on the companion cell side, which feed in to a single pore on the sieve element side of the wall [3] (Figure 1c,d). The plasmodesmata between companion cells and sieve elements are not well characterized, but they appear to be functionally distinct from those connecting mesophyll cells in leaves.

Mesophyll plasmodesmata have a low 'size-exclusion limit', allowing passive diffusion only of molecules less than 1 kDa, whereas dextrans of 10 kDa and a fluorescein-conjugated protein of 25 kDa have been found to pass freely from companion cells to sieve elements following microinjection [4,5]. Despite this high size-exclusion limit, most proteins and protein-containing complexes probably do not pass freely through plasmodesmata. Molecules below the size-exclusion limit might have signal sequences that cause their preferential accumulation in sieve elements. Furthermore, the three-dimensional configuration of a molecule might affect its movement potential. In the case of large proteins or protein–RNA complexes, the interaction with plasmodesmata most likely requires specific targeting signals or the ability to interact with plasmodesmata chaperones.

Pathogens use an endogenous transport pathway

For macromolecules to act in long-distance signaling in a plant, they must transit through plasmodesmata. Evidence for such movement of RNA and protein molecules first came from studies of plant pathogens. Proteins capable of interacting with plasmodesmata were discovered as virus-encoded 'movement proteins' [4]. Many such movement proteins localize to plasmodesmata in infected tissue and in transgenic plants. They undergo transport between cells, bind to nucleic acids and facilitate the transport of dextrans with molecular weights up to 20 kDa [4]. This last activity, known as 'gating', is believed to involve the movement-protein-mediated modification of plasmodesmata. Movement proteins presumably bind to their cognate viral genomes, mediating their transport into adjacent cells and subsequently — often with the aid of other viral proteins — into the vascular stream of the infected plant [6]. The first RNA molecule that was found to undergo cell-to-cell transport without a protein partner was the potato spindle tuber viroid. This non-translatable, self-replicating RNA

Figure 1



Anatomy of plant vascular tissue. **(a)** Diagram of the vascular tissue (magenta) within a plant. **(b)** Diagram of stem section showing vascular bundles. Vascular bundles are composed of xylem (red), responsible for water transport, and phloem (blue), responsible for the delivery of sugars, amino acids and macromolecules to all areas of the developing plant. **(c)** Transmission electron micrograph of a companion cell (CC) and adjoining sieve element (SE), connected by plasmodesmata (PD). Notice the high cytoplasmic content of the companion cell and the relative vacancy in the sieve element. (Image reproduced with permission from [18].) **(d)** Transmission electron micrograph of plasmodesmata connecting a companion cell (CC) to a sieve element (SE). (Image reproduced with permission from [19].)

presumably interacts with plasmodesmata to induce its own transport between cells [7]. The precise mechanism of viral/viroid movement remains unknown, but the speed at which it occurs suggests that it involves the hijacking of an endogenous cellular pathway.

A barrier to pathogen spread

Studies with plant viruses have demonstrated the uniqueness of plasmodesmata between companion cells and sieve elements. To cause a systemic infection, viruses must be transported through the phloem and infect new tissues. The genomes of some plant viruses encode a protein, in addition to movement protein(s), that acts, at least partially, to facilitate virus entry into the vasculature [6,8]. Virus entry into the phloem is often impeded, however, either because of natural limitations or as a result of some mutation of the virus or host genome. These restrictions are frequently host-specific, implying that different mechanisms or receptors control phloem access.

The luteoviruses and some geminiviruses are phloem-limited: they do not spread substantially into surrounding tissues after entry into the sieve elements by insect vector feeding [9,10]. The genomes of these viruses presumably do not encode proteins that allow their transport through the plasmodesmata that connect companion cells and sieve elements and into adjacent leaf tissue. Indeed, the phloem-limited geminivirus squash leaf curl virus avoids this impediment altogether by using tubules derived from the endoplasmic reticulum to span the cell wall and thereby infect adjacent cells [10]. These observations further suggest that the plasmodesmata between companion cells and sieve elements might act as a selective barrier for vascular transport.

Two host-plant components that appear to affect systemic virus movement — and by assumption phloem entry — have recently been identified in *Arabidopsis*. In *virus systemic movement* (*vsm1*) mutant plants, tobamoviruses are unable to move through the phloem, although their cell-to-cell movement is unaffected. The movement of an unrelated virus is unaltered in *vsm1* plants, suggesting that the lesion affects a host component involved in a specific virus–host interaction required for systemic spread [11]. The long-distance transport throughout host plants of tobacco etch virus is similarly blocked in plants carrying the ecotype-specific allele *RESTRICTED TEV MOVEMENT 1* (*RTM1*) [12]. The cloning and further characterization of the genes should shed light on the roles their products play in phloem-dependent movement of viruses and perhaps also of endogenous plant components.

Selective transport of endogenous molecules

The plasmodesmata between companion cells and sieve elements could play a role in regulating macromolecule movement throughout a plant. The presence of a multitude of proteins, and also several RNAs, in phloem exudates indicates that there is an efficient system for their transfer through companion cell plasmodesmata [1,13–15]. Some of the phloem proteins are found in sieve elements, while the RNAs that encode them occur predominately in companion cells, implying that proteins made in the companion cells can be specifically targeted for export to the sieve elements (Table 1).

To date, all phloem-exudate proteins and RNAs appear to be synthesized within the vascular tissue itself, suggesting a role in vascular differentiation or maintenance. In contrast, it is assumed that long-distance signaling molecules

Table 1

Potential endogenous plant transport proteins.

(a) Endogenous proteins that undergo cell-to-cell transport and that gate dextrans.

Protein	Protein size	Ascribed cellular function	RNA transported	Gating size limits	Reference
Thioredoxin h	13 kDa	Thioltransferase	N/A	9.4–20 kDa	[17]
Knotted	45 kDa	Homeobox transcription factor	Yes	20–40 kDa	[20]
Glutaredoxin	11 kDa	Thioltransferase	N/A	20–40 kDa	[13]
Cystatin	11 kDa	Cysteine protease inhibitor	N/A	20–40 kDa	[13]
PP2	24 kDa*	Phloem lectin	N/A	20–40 kDa	[13]
Exudate proteins	10–200 kDa	Unknown phloem proteins	N/A	20–40 kDa	[13]
CmPP16	16 kDa	Unknown	Yes	11 kDa	[2]

(b) Protein and mRNA molecules that are differentially distributed between companion cells and sieve elements.

Protein	Protein size	Ascribed cellular function	RNA localization	Protein localization	Reference
PP2	24 kDa*	Phloem lectin	CC	CC/SE	[21]
PP1	96 kDa	Phloem filament protein	CC	CC/SE	[22]
SUT1	47 kDa	Sucrose transporter	CC/SE PD†	SE	[23]
Thioredoxin h	13 kDa	Thiotransferase	CC	SE	[14,17]
CmPP16	16 kDa	Unknown	CC/SE‡	SE	[2]

(a) Proteins capable of moving between cells when microinjected into mesophyll cells, following purification and labeling with fluorescein. Gating for dextrans is monitored following co-injection of unlabeled protein and fluorescent dextrans of a particular size (kDa) into mesophyll cells. RNA transported indicates whether protein was tested for ability to transport RNA in *trans*. (b) Examples of mRNAs and proteins that are differentially localized between companion cells (CCs) and sieve elements (SEs), indicating that they are transported between the two types of cell through plasmodesmata (PD). *PP2 acts as a dimer of 48 kDa. †'SE PD' indicates that the RNA was localized to within plasmodesmata leading into sieve elements. ‡Found in immature sieve elements and companion cells, but predominately in mature companion cells. N/A, not assayed.

originate in non-vascular tissue and secondarily enter the phloem stream. No such signaling molecule has been documented, but some proteins — many specific to the phloem — have been found to undergo local transport through plasmodesmata and to cause an increase in the size-exclusion limit when microinjected into mesophyll cells (Table 1a).

Not all proteins in the phloem potentiate their own cell-to-cell movement, however, as fluorescently-labeled ubiquitin, an 8.5 kDa protein which accumulates in sieve elements, is unable to travel between mesophyll cells or to increase the size-exclusion limit on 20 kDa dextrans following microinjection into mesophyll cells [13]. This result suggests the need for 'chaperones' specific for the plasmodesmata between companion cells and sieve elements, which recognize the proteins or RNAs destined for transport, facilitate their transport into the phloem stream and perhaps also enable their delivery into the appropriate target tissue.

Phloem 'chaperones'?

A protein has recently been isolated from phloem exudate of *Cucurbita maxima* plants that behaves like a potential plasmodesmatal chaperone [2]. When microinjected into mesophyll cells this small (16 kDa) protein, known as CmPP16, is transported through plasmodesmata, facilitates the transport through plasmodesmata of dextrans and potentiates RNA movement. In companion cells, *CmPP16* mRNA is detected but not its protein product, whereas in enucleate sieve elements both the mRNA and protein are detected; this suggests that *CmPP16* mRNA and protein are both transported away from the companion cells through plasmodesmata [2]. The presence of CmPP16 in the phloem, and its apparent ability to facilitate transport of both its own and other RNA molecules, suggests that it might be an endogenous regulator of macromolecule transport through the phloem.

It is, however, possible that the *CmPP16* mRNA and protein molecules detected in enucleate sieve elements were not specifically exported from companion cells, but might rather be remnants from immature nucleated sieve elements. The microinjections were not performed in companion cells and the *CmPP16* mRNA and protein were not localized to within the plasmodesmata connecting the

companion cells to sieve elements [2]. Additional functional data are therefore required to determine whether CmPP16 has a role in RNA transport, where such transported RNAs originate and what effect they have on the receiving tissues.

Roles for transported molecules?

The discovery of specific RNA molecules in phloem exudates suggests that mRNAs may be transported between cells as components of a systemic signaling system, although neither the function nor the origin of these RNAs is known [14]. Additional evidence for such an RNA signaling system has come from studies of gene silencing, where virus or endogenous genes are repressed by the presence of homologous genes (often in the form of overexpressed transgenes). This 'post-transcriptional gene silencing' is thought to be mediated by an RNA-based mechanism of transcript destruction [16]. As post-transcriptional gene silencing results in silencing throughout the plant, the process might involve the transport of 'silencing RNAs' through the phloem. This potent antiviral defense mechanism might also act to regulate endogenous developmental or physiological programs. The identification of transported mRNAs and elucidation of their roles are required to further our understanding of the mechanism and physiological functions of such a transport system. The identification and detailed analysis of putative plasmodesmatal 'chaperones' capable of directing the phloem transport of RNA should provide insights into the systemic transport of RNA molecules in plants.

Function and regulation of a transport system?

It is increasingly likely that RNA and protein molecules — including complexes between the two — are transported both locally and systemically in plants. This raises myriad questions regarding the signals that they convey and how their targeting is controlled. For example, if these molecules alter the fate of target tissues, how is their transport regulated so that they are delivered to the right place and at the right time? Several movement proteins bind to single-stranded nucleic acids in a non-sequence-specific manner, and CmPP16 has been found to interact with several RNAs that have been tested [2,6]. Given this apparently lack of specificity, how do the putative chaperones identify the correct RNAs to be transported? One possibility is that they are translated close to their cargo, so that they are only ever allowed to contact the correct RNAs. To protect against the non-specific transport of all cellular RNAs, is seems likely that the molecules destined for transport are marked by having plasmodesmata-specific and/or phloem-specific signal sequences.

Are the transported RNAs translated in the receiving tissues, or do the RNA molecules themselves contain functional information? Evidence for the latter possibility has come from the recent observation that RNA3 of beet necrotic yellow vein virus, and not its protein product, facilitates vascular movement of the virus [17]. And what are the advantages of signalling via RNA molecules, rather than proteins? If plasmodesmata-specific and/or phloem-specific signal sequences exist, how do they compete with other signal sequences, such as nuclear localization signals, when within the same molecule? How are the putative macromolecular signals retrieved from the phloem stream? There are many reports of endogenous proteins and RNAs moving between cells, but how they function and what events they trigger in their target tissues remain elusive. Experimental systems that make it possible to determine the functions of transported proteins or RNAs in recipient tissues are essential. Clues from studying pathogen spread, post-transcriptional gene silencing and phloem transport should illuminate the complex mechanism of whole-plant signaling through plasmodesmata.

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